REMARKS

Claims 1, 4-35 and 38-67 are pending. Claim 12 is canceled. Claims 1, 35 and 62-67 are amended herein.

Claim 1 is amended to recite "wherein said amplification profile provides quantitative information regarding the abundance of said plurality of different amplification templates in said amplification reaction mixture at the start of said amplification regimen." The amendment language is supported, for example, in claim 12 as originally filed, and at page 8, lines 12-14.

Claim 35 is similarly amended to recite "wherein said comparing provides quantitative information regarding the abundance of said plurality of different amplification templates produced by said first and second entities." Support for the amendment language is found, for example, at page 17, lines 9-14.

Claim 63 is amended to recite "and wherein said profile provides quantitative information regarding the abundance, in said amplification reaction mixture, at the start of said amplification regimen, for said at least five nucleic acid species." Support in the specification for the amendment language is found, for example, at page 6, lines 17 to 19, at page 17, lines 9-14 and 19-27, and page 20, lines 1-4.

Claim 64 is amended to recite "wherein said amplification profile provides quantitative information regarding the abundance of said plurality of different amplification templates in said amplification reaction mixture at the start of said amplification regimen." Support in the specification for the amendment language is found, for example, at page 7, lines 5-17 and at page 8, lines 12-14.

Claim 65 is amended to recite "wherein said comparing provides quantitative information regarding the abundance of said plurality of different amplification templates produced by said first and second entities." Support in the specification for the amendment language is found, for example, at page 9, line 22 to page 10, line 19, at page 6, lines 17 to 19, at page 17, lines 9-14 and 19-27, and page 20, lines 1-4.

Claim 67 is amended to recite "and wherein said profile provides quantitative information

regarding the abundance, in said amplification reaction mixture, at the start of said amplification regimen, of said nucleic acid species." Support in the specification for the amendment language is found, for example, at page 6, lines 17 to 19, and at page 6, line 27 to page 7, line 4.

New claims 68 and 69 are added. New claim 68 is supported in the specification at, for example, page 7, lines 5-17, page 8, lines 12-14, and page 22, lines 13-24. New claim 69 is supported in the specification at, for example, page 9, line 22 to page 10, line 19, and page 22, lines 13-24.

Rejections under 35 U.S.C. §112, Second Paragraph:

Claims 1, 4-24, 28-34 and 64 are rejected under 35 U.S.C. §112, second paragraph as being confusing for lack of "a connecting clause such as 'comprising' after the preamble."

Claims 1 and 64 are amended herein to add the term "comprising" after the preamble. Reconsideration and withdrawal of this rejection under §112, second paragraph is respectfully requested.

Claims 63 and 67 are rejected under 35 U.S.C. §112, second paragraph as confusing "because it cannot be determined what is encompassed by 'determining the transcription profile' of a nucleic acid sequence." The Office Action states that "While this language is recited in the preamble and the final step of the claims, it is unclear what action(s) is required to satisfy this phrase."

Each of claims 63 and 67 is amended herein to replace the language "and determining the transcription profile of said nucleic acid sequence" with the language "whereby a transcription profile is determined for said nucleic acid species." Reconsideration and withdrawal of this rejection under §112, second paragraph is respectfully requested.

Rejection under 35 U.S.C. §112, First Paragraph:

Claims 1, 3-35 and 38-67 are rejected under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. The Office Action states:

> The arguments and declaration argue that capillary electrophoresis, as taught by Schumm et al., for example, cannot be used in or adapted to the method of Wiesner, substituting for the slab gel analysis of Wiesner. However, this appears to be exactly what applicant is currently claiming. In other words, the arguments and declaration appear to be arguing that the methods as currently claimed would not work, and thus are not enabled, since it is argued that capillary electrophoresis cannot be used to obtain quantitative information from aliquots of amplification reactions, and the instant claims are drawn to methods requiring this. (Emphasis in original)

Applicant respectfully disagrees.

Applicant has not argued that capillary electrophoresis cannot be used to obtain quantitative information from aliquots of amplification reactions. Applicant has argued, rather, that one of skill in the art cannot use capillary electrophoresis in the quantitative method described by Wiesner. Applicant argued previously that "in order to achieve the quantitation that is central to Wiesner's method, one must know the actual volume of sample applied to the separation and the absolute amount of amplified PCR product in a separated peak." Applicant, with support from Dr. Slepnev's declaration, argued that "because these values are not known for capillary electrophoresis, one of skill in the art cannot use capillary electrophoresis in the quantitative method described by Wiesner." That is, one cannot simply substitute capillary electrophoresis for slab gel electrophoresis in Wiesner's quantitative method, because two parameters critical for the quantitation will be missing. Applicant again notes that quantitation is central to the methods described by Wiesner.

Applicant has taken a different approach to quantitation than that taught by Wiesner, this approach permitting relative quantitative measurement of amplification templates *without* a requirement for knowledge of the volume of the separated aliquot *or* the absolute amount of amplified product in the separated peak. This is fundamentally different from the approach taught by Wiesner. That is, Applicant submits that this is not a simple adaptation of Wiesner to capillary electrophoresis, because neither Wiesner nor the combination references Schumm or Brenner teaches how to obtain relative quantitative measurement of amplification templates without knowledge of the volume of the aliquot separated or the absolute amount of amplified product in the separated peak.

Applicant submits herewith a second declaration of inventor Dr. Vladimir I. Slepnev under 37 C.F.R. 1.132, providing confirmation that quantitative analysis of amplification templates and amplification profiles can be reliably obtained through capillary electrophoresis of aliquots taken at plural stages during a PCR amplification reaction. In the declaration, Dr. Slepnev provides template quantitation data obtained using capillary electrophoresis of aliquots taken at various cycles of an amplification reaction, including a multiplex amplification reaction, as described in the specification. Dr. Slepnev explains in the declaration that the quantitative approach involves calculation of a threshold cycle, C_t, which permits the extrapolation of initial template abundance. The calculation of a C_t is described in the specification at page 22, lines 13-24. Dr. Slepnev explains in the new declaration that the approach described in the specification and employed to obtain the quantitative data provided in the declaration provides relative template concentration data. Thus, the quantitative data provided in Dr. Slepnev's declaration were obtained using methods supported in the specification.

Dr. Slepnev emphasizes in his second declaration that, "In stark contrast to what Wiesner teaches, we have devised a way to provide quantitative information regarding the initial abundance of template nucleic acids ***without*** *a requirement* for knowledge of the exact volume of the aliquot separated, and ***without*** *reliance* upon the ability to measure precisely the absolute quantity of a PCR product band at a given cycle." Dr. Slepnev states "Given the teachings of Wiesner, the ability to do so using capillary electrophoresis is surprising and not obvious."

Dr. Slepnev describes the approach that does not require knowledge of the aliquot volume or absolute quantity of PCR product band, noting in section 7 that "These data were obtained using methods as described in the specification, including the calculation of threshold cycles for the amplified species, and demonstrate that the invention works to provide quantitative information as presently claimed." That is, the data in the declaration confirm the ability to apply CE to plural aliquots withdrawn from a PCR reaction as an approach to determining the quantity of the target nucleic acids, without knowledge of the aliquot volume or the absolute amount of PCR product in a given band..

Dr. Slepnev explains the relationship between the initial number of DNA copies in a reaction, the efficiency of PCR amplification and the amount of product at a given cycle, C in

equation 1: $Q_{(C)}= Q_0 * E^C$, wherein $Q_{(C)}$ is the amount of DNA copies at cycle C, Q_0 is the initial number of DNA copies in the reaction and E is efficiency of PCR amplification (number of DNA molecules generated per PCR cycle), at section 8 of the declaration. He further explains the relationship of the threshold cycle (Ct), as follows:

> Kinetic PCR (such as for example, real-time PCR) uses transformed equation 1 to calculate number of cycles required to generate a fixed (threshold) number of DNA copies.
>
> $T= Q_{(C)}=const$
>
> $LogT= \log Q_0 + C_t \log E$ C_t=cycle at threshold T
>
> $Ct= const- (1/\log E)*\log Q_0$
>
> If one can use experimental methods to assess quantity of DNA wherein the measured signal F is directly proportional to the number of DNA copies in the reaction $Q_{(C)}$,
>
> $F=f* Q_{(C),}$
>
> then threshold value can be set in the measured experimental units.

Dr. Slepnev goes on to state that

> "Real-time PCR employs measurement of fluorescent signal (generated by fluorescent probes interacting with amplified DNA) to calculate the number of cycles required to reach the arbitrary selected threshold of fluorescent signal as a function of initial number of DNA copies in the reaction Q_0. By varying the Q_0 and measuring the Ct corresponding to the given Q_0, one can create a calibration plot and determine copy number in the unknown sample by measuring Ct and correlating it to the Q_0 in the calibration plot."

Dr. Slepnev explains the basic assumptions under which one can apply capillary electrophoresis to determine an initial abundance of template nucleic acid in a reaction. These are:

> a) that the quantitative fluorescent signal (such as peak height or peak area) is proportional to the amount of DNA molecules; and
>
> b) that the amount of injected (and therefore detectable DNA molecules) is proportional to the amount of DNA in the PCR reaction.

Dr. Slepnev explains that the first condition requires a consistent way to detect peaks on electrophoregrams, and that the second condition, which is particularly important for electrokinetic injection during CE, assumes that changing representation of different DNA species (such as PCR primers and PCR products) during PCR does not *considerably* affect their injection properties. Dr. Slepnev states that "The validity of these assumptions was demonstrated experimentally" and provides data to support this statement in the declaration.

Dr. Slepnev summarizes the steps involved in generating the data provided in the declaration, stating that the methods "encompass performing PCR amplification, taking a series of aliquots of the PCR reaction (e.g., at every cycle or every other cycle), separating amplified DNA by CE, determining the Ct (number of cycles to reach the threshold fluorescence value selected as log of peak height or peak area expressed in relative fluorescent units) that can be measured for multiple DNA targets simultaneously."

Dr. Slepnev states that "by applying different known amounts of DNA targets Q_0 and measuring the Ct corresponding to the given Q_0, one can create a calibration plot and determine copy number in the unknown sample by measuring Ct and correlating it to the Q_0 in the calibration plot."

Dr. Slepnev's declaration includes data obtained from a multiplex reaction using known amounts of viral nucleic acids representing Cytomegalovirus (CMV), BK virus (BK), and Human Herpesvirus 6 (HHV6B) and Human Herpesvirus 7 (HHV7). In section 6(A) of the declaration, Dr. Slepnev explains that the viral nucleic acids were mixed such that each viral nucleic acid was at a different copy number, in order to demonstrate the linearity of the system. To mimic an actual determination from, for example, a clinical sample, the nucleic acids were spiked into a plasma sample and extracted to prepare templates for the amplification.

In section 6(B) of the declaration, Dr. Slepnev explains the cycling regimen and the sampling process by which aliquots of the reaction are withdrawn from the amplification reaction mixture. He further explains the electrokinetic injection of withdrawn aliquots into the CE apparatus, and the separation and detection of amplified products. Dr. Slepnev explains that "the fluorescent intensity data for peaks corresponding to target amplicons were extracted from

GeneMapper Software (Applied Biosystems), plotted versus cycle number and subjected to linear regression analysis to identify exponential phase and to calculate Threshold Cycles for target nucleic acids."

In section 6(C) of the declaration, Dr. Slepnev explains the approach taken for data analysis and template quantitation. The method identifies a Ct for each viral target template, and plots a calibration plot of Ct versus cycle number for each viral target based on known concentration of the viruses. Dr. Slepnev explains that the generated standard or calibration plots "were used for quantification of the set of samples in which viruses were spiked at the different copy number (the measured Ct for the sample was correlated to the log Q0 using a calibration plot for corresponding virus)." In confirming the accuracy of the template measurement, he explains that "The expected copy number was plotted versus measured copy number demonstrating excellent correlation between two values and validating the use of the described multiplex quantification of target viruses," referring to appendix A, figure 2. Dr. Slepnev again emphasizes that the initial abundance of template nucleic acid was determined without needing to know the volume of the aliquot separated or the absolute quantity of the PCR product bands:

> "It is emphasized that initial abundance of template nucleic acids was determined *without* a requirement for knowledge of the *exact volume* of the aliquot separated, and *without reliance* upon the ability to measure precisely the *absolute quantity* of a PCR product band at a given cycle."

Dr. Slepnev also provides, in appendix B, a copy of a published manuscript establishing that the methods as claimed can be applied in a reverse-transcription PCR approach. He states that "The method used followed the same steps outlined in paragraph 6, with few modifications," noting that "the reverse transcription step preceded the PCR amplification, the CE analysis was performed on ABI 3100 instrument system using Gene Scan data analysis software (Applied Biosystems), and the Cts were calculated as extrapolation of experimental data from plots of Ct versus input RNA copy number to the log peak area=3." Dr. Slepnev states that the manuscript "further demonstrates a method in which initial template amounts are determined without knowledge of the exact volume of the aliquot separated, and without reliance upon the ability to measure precisely the absolute quantity of a PCR product band at a given cycle."

In view of the confirmation in Dr. Slepnev's declaration that the methods described and

claimed in the instant application work to provide quantitative information on template concentrations from multiplex amplification reactions, using capillary electrophoresis, but without knowledge of separated aliquot volume or knowledge of the amount of amplified nucleic acid in a separated peak, Applicant submits that the invention as claimed is fully enabled. Reconsideration and withdrawal of the rejection under §112, first paragraph is respectfully requested.

In view of the above, all issues raised in the Office Action have been addressed herein. Entry of the amendments and consideration of the claims is respectfully requested.

Respectfully submitted:

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